Bisphenol A and Reproductive Health: Update of Experimental and Human Evidence, 2007–2013

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Abstract

Background: In 2007, an expert panel reviewed associations between bisphenol A (BPA) exposure and reproductive health outcomes. Since then, new studies have been conducted on the impact of BPA on reproduction.

Objective: This review summarizes the data obtained since 2007, focusing on: 1) findings from human and animal studies, 2) the effects of BPA on a variety of reproductive endpoints, and 3) mechanisms of BPA action.

Methods: We reviewed the literature using a PubMed search from 2007-2013 based on keywords related to BPA and male and female reproduction.

Discussion: BPA is an ovarian toxicant because it affects the onset of meiosis in both animal and *in vitro* models, interferes with germ cell nest breakdown in animal models, accelerates follicle transition in several animal species, alters steroidogenesis in multiple animal models and women, and reduces oocyte quality in animal models and women undergoing IVF. BPA is a uterine toxicant because it impairs uterine endometrial proliferation, decreases uterine receptivity, and increases implantation failure in animal models. BPA exposure may be associated with adverse birth outcomes, hyperandrogenism, sexual dysfunction, and impaired implantation in humans, but additional studies are required to confirm whether this is the case. BPA is a testicular toxicant in animal models, but the data in humans are equivocal. Finally, insufficient evidence exists regarding effects of BPA on the oviduct, placenta, and pubertal development.

Conclusion: BPA is a reproductive toxicant because it impacts female reproduction, and has the potential to affect male reproductive systems in humans and animals.

Introduction

Bisphenol A (BPA) is a high production volume chemical used in a variety of commonly used consumer products. Most notably, BPA is present in polycarbonate plastics, the epoxy resin liners of aluminum cans, and thermal receipts (Ehrlich et al., 2014). BPA is an endocrine disrupting chemical that can act through a variety of physiological receptors such as genomic estrogen receptors 1 and 2, membrane bound estrogen receptors, androgen receptor, peroxisome proliferator-activated receptor gamma, and thyroid hormone receptor (reviewed in Richter et al. 2007).

In 2006, to address research gaps in the published BPA studies, an organized committee sponsored by the National Institute of Environmental Health (NIEHS), National Institute of Dental Craniofacial Research (NIDCR), United States Environmental Protection Agency (US-EPA), and Commonweal reviewed the associations between BPA exposure and reproductive health outcomes and published their analyses in 2007 (vom Saal et al. 2007). This committee was organized into five topic-driven panels that evaluated the available data and developed guidelines for the conduct of future *in vivo* and *in vitro* studies that would facilitate comparisons across studies and extrapolations to human health outcomes. Overall, the panels recommended the use of oral and dietary exposure routes, doses of BPA similar to human exposure, and further evaluations of BPA concentrations in animal and human tissues and fluids with the aim of using doses that would result in human-relevant serum concentrations of unconjugated BPA in studies with experimental animals.

With respect to reproductive effects, the subpanel evaluating *in vivo* animal studies found contradictory results among studies but concluded that, based on the available evidence, they were confident that BPA impacts the male reproductive system and thought it likely, but

requiring confirmation, that both developmental and adult exposure affects the female reproductive system (Richter et al. 2007). At the time, the prostate was the most extensively studied reproductive tissue, with both developmental and adult exposures reported to increase prostate size (reviewed in Richter et al. 2007 and vom Saal et al. 2007). However, not all studies reported effects, and controversy remained regarding the doses necessary to elicit effects. In females, effects on the oocyte, developing reproductive tract, and timing of sexual maturation were available, but many remained largely unconfirmed (reviewed in Richter et al. 2007 and vom Saal et al. 2007). Similarly, very limited epidemiological information was available, and the few published studies were limited in their design, restricting the strength of the conclusions that could be drawn about the reproductive hazards of BPA exposure. Nevertheless, the available evidence suggested an association between BPA exposure and polycystic ovarian syndrome (PCOS) (reviewed in Richter et al. 2007 and vom Saal et al. 2007).

In the intervening six years, new studies on the impact of BPA on the reproductive system have been conducted and are summarized in this review. Specifically, this review focuses on summarizing: 1) reproductive findings from human studies and how they compare with animal studies, 2) detailed information about the experimental effects of BPA on a variety of reproductive endpoints, taking into account species, dose, route of exposure, and timing of exposure, and 3) mechanisms of BPA action in the reproductive system whenever possible. Thus, this update is a compilation of the more recent literature detailing the effects of BPA exposure on the female and male reproductive systems. The insights and conclusions of this review should be used as directors for future studies and to develop consensus statements about the effects of BPA on the reproductive system based on the state of the evidence.

Methods

Search strategy

We performed a Pubmed search to identify journal articles related to BPA and reproduction between 2007 and 2013. Search terms included ovary, follicle, oocyte, oocyte competence, meiosis, granulosa cell, theca cell, ovulation, oviduct, uterus, endometrial, stroma, implantation, pregnancy, miscarriage, spontaneous abortion, embryo, blastocyst, placenta, trophoblast, cytotrophoblast, syncytiotrophoblast, birth, birth weight, gestation, transgeneration, testes, sperm, Leydig cell, Sertoli cell, germ cell, epididymis, prostate, ventral lobe, dorsal lobe, ejaculation, fertilization, infertility, sexual dysfunction, estradiol, testosterone, androstenedione, estrone, dihydrotestosterone, dehydroepiandrosterone, and pregnenolone. As a final term, after the aforementioned search, searches of bisphenol A and BPA were used to identify any papers without the other selected search terms or term variants.

Article inclusion criteria

Journal articles that were found using the search terms and in the time-frame were considered for inclusion. All human and experimental animal *in vivo* studies were included. *In vitro* studies were included when there was a clear mechanism for an effect or if the studies supported *in vivo* or human findings. Journal articles were not omitted due to a paucity of research in any category.

Dose designation

Similar to the 2007 expert panel report and to Vandenberg et al. (2013), we defined "low dose" in this review as BPA doses at or below 50 mg/kg/day. This is the currently accepted lowest adverse effect level (LOAEL) used by the EPA (EPA 1993). Thus, "high dose" BPA studies are those with concentrations above 50 mg/kg/day. Throughout the text, we use the terms "low dose"

and "high dose" to provide a simple description of the doses used in each study. However, specific doses used in each study are listed in each supplemental table.

Exposure timing

Throughout the review, gestational exposure is when BPA exposure occurred *in utero*, neonatal exposure is BPA exposure after birth but before weaning, and postnatal exposure is BPA exposure any time after weaning.

Article strength determination

Similar to the 2007 expert panel report, we considered the evidence to be strong when multiple studies in multiple species indicated a similar effect of BPA on a reproductive tissue or endpoint, even if concordance was not 100% across all studies given that species and strain differences can lead to differences in dose response and magnitude of effect. We considered the evidence to be limited, when some, but not the majority of studies indicated a similar effect of BPA on a reproductive tissue or endpoint and/or when data from *in vitro, in vivo* animal, and human studies were discordant. Finally, we considered the evidence to be inconclusive when a limited number studies were done to examine the effect of BPA on a selected reproductive tissue or endpoint and/or when the studies were only conducted in one species or with *in vitro* studies alone. We recognize that *in vitro* studies have played critical roles in identifying how BPA affects specific tissues, but it was often difficult to correlate human and animal health outcomes with *in vitro* outcomes alone, leading us to classify studies as inconclusive if they were only conducted *in vitro*.

Early oogenesis and ovarian follicle formation

All studies on BPA exposure and early oogenesis and follicle formation have been conducted using animal models or *in vitro* systems. These studies indicate that developmental BPA exposure has the potential to affect two stages of oogenesis: 1) the onset of meiosis in the fetal ovary and 2) germ cell nest breakdown and follicle formation (Supplemental Material, Table S1). Several reports have confirmed the original findings from studies in mice (reviewed in Richter et al. 2007) that gestational exposure affects the onset of meiosis and induces nondisjunction in meiosis in the fetal ovary, but does not induce aneuploidy. In a macaque study designed to mimic serum levels of unconjugated BPA reported in human biomonitoring studies (Vandenberg et al. 2010), daily low dose BPA exposure (measured <1 ng/ml in maternal serum) significantly disrupted synapsis and recombination between homologous chromosomes at the onset of meiosis (Hunt et al. 2012), consistent with previous findings (Susiarjo et al. 2007). In three studies, gestational low dose BPA exposure of mice induced changes in gene expression in germ cells and early meiocytes. BPA exposure increased expression of *Stra8* (stimulated by retinoic acid 8 homolog) and a variety of meiotic genes in C57BL/6 mice (Lawson et al. 2011). However, longer gestational BPA exposure down-regulated the expression of *Stra8*, *Dazl* (deleted in azoospermia-like), and *Nobox* (newborn ovary homeobox) in CD-1 mice (Zhang X et al. 2012). Lastly, BPA exposure commencing after the onset of meiosis induced a meiotic delay at gestational day (GD) 17.5 in CD-1 females (Zhang H et al. 2012). Collectively, these studies provide strong evidence that BPA exposure disrupts meiosis in mice and macaques, as well as alters gene expression in germ cells and early meiocytes in two different strains of mice.

In vitro studies provide further evidence that BPA impacts the onset of meiosis. BPA (1 to 30 μM) increased oocyte degeneration by impairing meiotic progression in cultured human fetal

oocytes and, similar to mouse studies, human fetal oocytes that progressed to prophase exhibited increased levels of recombination (MLH1 foci) and gene expression changes (Brieño-Enriquez et al. 2011a, 2011b). BPA also increased methylation errors in differentially methylated regions of maternally imprinted genes of oocytes in cultured preantral follicles of C57/BL6xCBA/Ca mice (Trapphoff et al. 2013). Thus, the data from different mouse strains, macaques, and *in vitro* studies consistently provide strong evidence to conclude that BPA exposure has detrimental effects on the meiotic process both at the gene expression and phenotypic level.

Studies also suggest that BPA interferes with germ cell nest breakdown in animal models. In neonatally exposed lambs, low dose BPA increased the incidence of multi-oocyte follicles (Rivera et al. 2011). Similarly, in gestationally exposed macaques, dietary low dose BPA exposure increased the number of oocytes present in secondary and antral follicles at birth and continuous BPA exposure (measured <1 ng/ml in maternal serum) increased the incidence of unenclosed oocytes (Hunt et al. 2012). Further, in gestationally exposed CD-1 mice, low dose BPA increased the number of unenclosed oocytes, while it decreased the number of primordial follicles in a dose-dependent manner (Zhang H et al. 2012). In another study, prenatal BPA exposure altered the fetal ovarian steroidogenic gene and microRNA expression that mediate gonadal differentiation and folliculogenesis in sheep (Veiga-Lopez et al. 2013). Collectively, these studies provide strong evidence that gestational BPA exposure across multiple exposure routes, doses, and species, impairs proper germ cell nest breakdown, leading to the formation of multi-oocyte follicles. The presence of multi-oocyte follicles is of concern because they are considered a pathologic condition that may lead to ovulatory problems (Iguchi et al. 1990).

BPA exposure also appears to accelerate follicle transition and growth in several species. Neonatal BPA exposure accelerated follicle transition in lambs, decreasing primordial and

increasing primary follicle numbers, without affecting total follicle numbers (Rivera et al. 2011). A similar enhanced activation of follicular recruitment was observed in neonatally exposed Wistar rats (Rodríguez et al. 2010). BPA exposure also increased cell proliferation, indicative of follicular growth, in small antral follicles in neonatally exposed lambs and Wistar rats (Rivera et al. 2011; Rodríguez et al. 2010). Taken together, the data suggest that BPA enhances the recruitment and growth of primordial and primary follicles across species. Combined with the effects on germ cell nest breakdown, there is strong evidence that BPA induces ovotoxicity by acting on developing and immature follicle stages in animals models. However, the consequences of these effects on reproductive potential/longevity are unclear. In one study, low dose neonatal BPA exposure decreased numbers of all follicle types and increased atretic follicles in rats during adulthood (Li Y et al., 2013). These effects of BPA on follicles could lead to premature reproductive senescence, but this needs to be confirmed in future studies.

In vitro studies on the effects of BPA have focused on mature ovarian follicles. In murine preantral follicles, BPA (3nM) accelerated development to antral follicles (Trapphoff et al. 2013). In murine antral follicles, exposure to BPA (440μM) aberrantly up-regulated expression of cell cycle regulators and the pro- and anti-atretic factors *Bax* (BCL2-associated X protein), *Trp53* (tumor protein 53), and *Bcl2* (B-cell lymphoma 2), inhibiting follicle growth and inducing apoptosis (Peretz et al. 2012). Further, BPA (110-438μM) inhibited antral follicle growth in mice (Ziv-Gal et al. 2013). These studies suggest that low dose BPA exposure may alter follicle formation, but high dose BPA may directly inhibit growth, cause atresia, and induce changes in gene expression in rodent antral follicles. In future studies, it will be important to validate findings from these *in vitro* studies at the *in vivo* level and determine the consequences of BPA-induced follicle toxicity on reproduction function.

Steroidogenesis in females

Multiple studies have investigated the association between BPA exposure and ovarian steroid hormone production in women (Supplemental Material, Table S2). In three publications based on women undergoing *in vitro* fertilization (IVF), BPA exposure was associated with a decrease in peak serum estradiol levels prior to oocyte retrieval (Ehrlich et al. 2012b; Mok-Lin et al. 2010; Bloom et al. 2011a). Additionally, in a case-control study, BPA was associated with increased testosterone and androstenedione levels in women with PCOS (Kandaraki et al. 2011). In a study of 60 women undergoing IVF, urinary BPA concentrations were not associated with a negative linear dose-response association with the expression of the steroidogenic enzyme *Cyp19* in granulosa cells collected at the time of oocyte retrieval, but instead a suggested non-monotonic dose-response association (Ehrlich et al. 2013). Conversely, BPA was not associated with estradiol or testosterone levels in women in the INChianti study, a prospective, population based study of adults living in Chianti, Italy (Galloway et al. 2010). Given the limited information on BPA exposure and ovarian steroidogenesis in women and the discrepant study results, additional studies utilizing sensitive and reliable steroid hormone and BPA assays are required to delineate whether BPA levels negatively impact reproductive hormonal patterns in women. Further, given that most existing studies on BPA exposure and steroid levels were conducted in IVF populations, it is critical to examine the association between BPA exposure and steroidogenesis in women from the general population.

Several experimental studies have examined the effect of BPA exposure on ovarian steroidogenesis in laboratory animals (Supplemental Material, Table S1). In three rodent studies, perinatal (Xi et al. 2011) and postnatal (Fernández et al. 2010; Tan, et al. 2013) low dose BPA exposure increased serum estradiol levels. Further, in one Sprague-Dawley rat and one pregnant

ICR mouse study, low dose BPA increased testosterone and progesterone levels (Fernández et al. 2010; Tan et al. 2013). Interestingly, in the Xi et al. study (2011), postnatal BPA exposure alone did not affect serum hormone levels in the mice. Similarly, in other studies using rats, mice, and lambs, gestational and/or gestational and neonatal BPA exposure had no effect on steroidogenesis (Kobayashi et al. 2012; Mendoza-Rodríguez et al. 2011; Rivera et al. 2011;Varayoud et al. 2011). In these studies, the doses used were lower than those in the affected studies, indicating that BPA doses less than 20mg/kg may not increase hormone production in animal models. In contrast, though BPA did not alter estradiol levels, low dose BPA decreased progesterone levels in adult mice during early pregnancy (Berger et al. 2008). Additionally, in adult rats, low dose BPA (below 0.1 mg/kg/day) decreased estradiol, testosterone, *Cyp19* (aromatase), and *Star* (steroidogenic acute regulatory protein) (Lee SG et al. 2013). Further, in adult mice, low dose BPA decreased expression of estrogen and progesterone receptors, though BPA did not alter hormone levels (Berger et al. 2010). The differences in study results may be a function of exposure times, internal doses of BPA, or species.

The results of *in vitro* studies on the effects of BPA on steroidogenesis are also equivocal. BPA exposure (44 and 440μM) inhibited estradiol, testosterone, androstenedione, estrone, dehydroepiandrosterone, and progesterone production, and decreased *StAR* and *Cyp11a1* (cytochrome P450 side-chain cleavage) expression in cultured intact murine antral follicles (Peretz et al. 2011; Ziv-Gal et al. 2013). However, in isolated rat theca-interstitial cells, BPA (100 nM to 100 μM) had opposite effects, increasing testosterone synthesis and *Cyp17a* (cytochrome P450 17alpha hydroxylase/lyase), *Cyp11a1*, and *StAR* expression (Zhou et al. 2008). In isolated rat granulosa cells, BPA (100 μM) decreased progesterone synthesis and increased *StAR* expression (Zhou et al. 2008). In a separate study using porcine granulosa cells,

0.1 μM BPA increased estradiol levels, while higher doses (1 and 10 μM) decreased estradiol levels. All three concentrations of BPA decreased progesterone levels (Grasselli et al. 2010). Collectively, these studies indicate that BPA adversely affects steroidogenesis *in vitro*, but the effects depend on BPA concentration, and that different levels of BPA have different effects on steroidogenesis depending on whether intact follicles or isolated cells are used in the cultures.

Oocytes: quantity, quality, and fertilization

A few human studies have analyzed the association between urinary BPA levels and oocyte yield, maturation, and fertilization (Supplemental Material, Table S2). A small prospective study of 58 infertile women and 37 male partners undergoing intracytoplasmic sperm injection (ICSI) or conventional IVF found an association between serum BPA concentrations and oocyte maturation only among Asian women, but an overall correlation between increasing serum concentrations and the developmental potential of human oocytes (Fujimoto et al. 2011). In two publications from the same prospective cohort of 84 women (Mok-Lin et al. 2010) and 174 women (Ehrlich et al. 2012b) undergoing IVF, increasing urinary BPA concentration was associated with decreased numbers of retrieved oocytes, mature oocytes (MII), and normally fertilized oocytes (2PN). These results suggest that BPA is associated with impaired oocyte yield, maturation, and fertilization, adversely affecting the success of IVF treatment.

Several recent experimental results support the findings from earlier studies that suggested BPA exposure affects the resumption of meiosis in the periovulatory oocyte (reviewed in vom Saal et al. 2007; Supplemental Material, Table S1). Neonatal low dose BPA exposure inhibited germinal vesicle breakdown (GVBD) in CD-1 F1 hybrid female mice (Chao et al. 2012), confirming the previous work of Hunt et al (2003). Conversely, low dose BPA administered to MF-1 (C57BL/6 x CBA/Ca F1 hybrid) mice from PND22 to PND28 did not reduce GVBD and polar body

extrusion or increase spindle aberrations (Eichenlaub-Ritter et al. 2008). Similarly, low dose BPA given orally to 4 or 9 week old superovulated female C57Bl/6 mice did not affect oocyte retrieval, meiotic maturation, or induce aneuploidy (Pacchierotti et al. 2008). While the exact reasons for the subtle discrepancies among studies are unknown, there are numerous possible reasons for the variation (reviewed in Hunt et al. 2009), including the window of neonatal exposure and potential co-exposure to phytoestrogens that may modulate the effects of BPA on the periovulatory oocyte (Muhlhauser et al. 2009).

In *in vitro* studies, BPA (43.8 μM) significantly altered spindle formation, distribution of pericentriolar material at spindle poles, and induced congression failure in MF-1 mouse oocytes isolated from antral follicles (Eichenlaub-Ritter et al. 2008). Additionally, BPA (30 µM) impaired spindle alignment and caused meiotic arrest after GVBD, but prior to polar body extrusion in follicle-enclosed oocytes from adult mice (Lenie et al. 2008). Importantly, a recent study of human oocytes exposed during *in vitro* maturation, with doses of BPA within the range measured in human follicular fluid (20, 200 ng/ml, and 20 µg/ml), found a dose-dependent increase in the incidence of meiotic arrest, disturbances in spindle formation and chromosome alignment, and spontaneous oocyte activation (Machtinger et al. 2013). Because the doses used were in the range measured in human follicular fluid, these data together with the results of experimental studies, provide compelling evidence that BPA adversely affects the maturing oocyte.

Polycystic ovarian syndrome (PCOS)

Studies on the effects of BPA on polycystic ovarian syndrome (PCOS) are limited (Supplemental Material, Table S2). Women with PCOS are characterized by oligo-anovulation, functional hyper-androgenism, and multi-follicular ovaries (accumulation of several small sized antral

follicles), with the majority of women showing insulin resistance and luteinizing hormone excess (Hampton 2013). One case control study (71 women with PCOS and 100 women without PCOS) reported an association between serum BPA levels and increased testosterone, androstenedione levels and insulin resistance in PCOS (Supplemental Material, Table S2; Kandaraki et al. 2011). Given the limited number of studies assessing the association between BPA exposure and PCOS symptoms, more studies are needed before firm conclusions can be made about the impact of BPA exposure on PCOS or PCOS symptoms.

The ovarian phenotype in BPA-treated rodents (cystic appearing follicles) differs from the ovarian phenotype of women with PCOS (accumulation of small antral follicles). In rodents, pre- and neonatal low dose BPA exposure lead to disruption of estrous cyclicity (Adewale et al. 2009), increased testosterone production (Fernández et al. 2010), and ovarian cysts (Newbold et al. 2009) (Supplemental Material, Table S1). High dose BPA exposure also leads to ovarian cysts (Fernández et al. 2010) and an accumulation of large antral follicles (Adewale et al. 2009). Additionally, BPA exposure decreased GnRH levels measured from hypothalamic explants *in vitro* (Fernández et al. 2010). Additional studies in other animal models are needed to fully understand whether BPA exposure causes PCOS or PCOS-like conditions and to determine why outcomes differ in animal models and women.

Oviduct

Only one study investigated the effects of BPA on the oviduct (Supplemental Material, Table S1). This study demonstrated that prenatal low dose BPA exposure causes progressive proliferative lesions in the oviducts of the offspring of CD-1 mice (Newbold et al. 2009). Because alterations in oviduct morphology would be expected to adversely affect both

fertilization and embryo transport, future studies are clearly warranted to examine the impact of BPA not only on the oviduct, but also on fertilization and embryo transport.

Uterine morphology

While no studies have reported the impact of gestational BPA exposure on uterine morphology in women due to the extreme difficulty of monitoring adult outcomes of prenatal exposure to BPA, animal studies suggest that gestational BPA exposure perturbs uterine gross morphology in the adult (Supplemental Material, Table S3). Specifically, low dose BPA exposure induces benign and malignant lesions (Newbold et al. 2009; Signorile et al. 2010) and endometrial polyps in the uterus as well as perturbs Wölffian duct regression in gestationally exposed, adult mice (Newbold et al. 2009). Further, adult hens exposed in ovo on day 4 of incubation to BPA (134 ng/kg) had decreased thickness of their tunica mucosa and density of uterine glandular structures compared to unexposed hens (Yigit and Daglioglu 2010). Together, these studies indicate that gestational BPA exposure may be potentially deleterious to uterine morphology in adult females. Importantly, since abnormalities have been identified in middle age that were not observed in young adults (Newbold et al. 2009), future studies should assess effects in exposed animals at different life stages.

Uterine endometrium

Limited data exist on BPA exposure and uterine endometrium in women. One case control study of 69 women suggests that serum BPA concentrations may be associated with the occurrence of endometriosis (Supplemental Material, Table S2) (Cobellis et al. 2009). Another study of 495 individuals in an operative cohort and 131 women in population cohort found no association between BPA exposure and endometriosis (Buck Louis et al. 2013). However, this study was not originally intended to investigate BPA exposure, was not appropriately powered to assess

endometriosis, and included a lapse between sample collection and endometriosis evaluation, further confounding these data. Given the lack of studies on BPA and endometrial disorders in humans, more studies are required before making conclusions about whether BPA adversely impacts the uterine endometrium in women.

Experimental studies support the findings from the aforementioned human study (Supplemental Material, Table S3). Adult female Balb-c mice exposed gestationally and neonatally to low dose BPA developed endometrial like structures with glands and stroma in adipose tissue surrounding the genital tract. These structures expressed *Hoxa10* (homeobox A10), a transcription factor that mediates proliferation of stromal tissue prior to implantation (Signorile et al. 2010). Both low and high dose BPA increased expression of *Hoxa10* in gestationally exposed adult CD-1 and ICR mice as well (Bromer et al. 2010; Hiyama et al. 2011).

Other *in vivo* studies provide evidence that BPA impairs proliferation in the uterus. BPA exposure over a wide range of doses, times, and routes decreased expression of uterine *Esr1* (estrogen receptor alpha) in the rodent, which may lead to inhibited endometrial proliferation in the uterine epithelium and stroma (Berger et al. 2010; Bromer et al. 2010; Varayoud et al. 2008; Bosquiazzo et al. 2010). In two separate studies of adult rats, gestational and neonatal low dose BPA exposure decreased uterine epithelium proliferation in response to hormone treatments (Varayoud et al. 2008; Mendoza-Rodríguez et al. 2011). While low dose BPA did not affect progesterone receptor expression, it dampened glandular and stromal progesterone receptor expression in response to estradiol stimulus in another (Aldad et al. 2011). Low dose BPA also impaired apoptosis of the uterine epithelium during estrus in neonatally exposed, adult rats (Mendoza-Rodríguez et al. 2011).

In vitro studies also support the hypothesis that BPA exposure adversely impacts the uterus. BPA (50 and 100 µM) significantly decreased proliferation of human endometrial stromal fibroblasts cultured for 48h (Aghajanova et al. 2011). BPA (50 µM) was also found to decrease the proliferation of cultured human endometrial endothelial cells (Bredhult et al. 2009). Further, in cultured, primary heterogeneous populations of uterine cells, BPA (10 µM) significantly inhibited uterine cell contractions, increased oxytocin-related pathways, and decreased prostaglandin-related signaling after 48h (An et al. 2013). Taken together, the existing animal and *in vitro* studies provide strong support that BPA impairs uterine cell proliferation.

Uterine receptivity and implantation

Only one study has reported on the association between BPA exposure and uterine receptivity/implantation in women (Supplemental Material, Table S2). In 137 women undergoing IVF, higher quartiles of urinary BPA concentrations were associated with increased odds of implantation failure (Ehrlich et al. 2012a). Given the limited information on this topic, future studies need to be conducted to determine whether BPA exposure is associated with adverse uterine receptivity/implantation outcomes in women.

Several experimental studies indicate that BPA exposure impairs uterine receptivity and implantation (Supplemental Material, Table S3). Repeated exposure of pregnant mice to low dose BPA during early gestation completely ablated embryo implantation (Berger et al. 2008; Xiao et al. 2011). A single exposure to high dose BPA on GD0 or GD1, but not on GD2 was found to decrease implantation in mice (Berger et al. 2008, 2010). Neonatal low dose BPA exposure also decreased implantation sites in pregnant rats (Varayoud et al. 2011). Interestingly, low dose BPA increased pre-implantation loss in unexposed females mated to neonatally exposed male rats (Salian et al. 2009a) and decreased implantation sites in females mated to

adult exposed male rats (Tiwari and Vanage 2013). Further, when untreated and healthy embryos were transplanted into the uteri of low dose BPA exposed mice, BPA prevented implantation (Xiao et al. 2011). Together, these studies provide strong evidence that BPA exposure, in both males and females, affects uterine receptivity in females. However, a need still exists to explore whether BPA-exposed embryos attach to the uterine epithelium and initiate implantation.

Neonatal low dose BPA exposure also decreased pregnancy maintenance in experimental studies (Varayoud et al. 2011). Low dose BPA increased resorption rates in uteri of unexposed females mated to either neonatally exposed male rats (Salian et al. 2009a), gestationally and neonatally exposed male rats (Salian et al. 2009b), and adult exposed rats (Tiwari and Vanage 2013), suggesting that male exposure can significantly contribute to pregnancy loss. These effects were also evident in F1 and F2 rat offspring (Salian et al. 2009a, 2009b), suggesting transgenerational effects of BPA. The potential ability of BPA to cause transgenerational effects in animal models is further supported by Hiyama et al (2011) who showed that developmental high dose BPA exposure reduced uterine weight, expanded the uterine lumen, and induced demethylation of the *Hoxa10* gene in the F2 generation in mice. Collectively, these experiments indicate that BPA exposure causes adverse effects on implantation and pregnancy maintenance in animal models, which may be transgenerational in nature.

Embryo development

Few studies have examined the effects of parental BPA levels on subsequent *in vitro* embryo development in humans (Supplemental Material, Table S2). In one prospective preconception cohort study of 174 women, total urinary BPA concentration was associated with a decreased rate of blastocyst formation (Ehrlich et al. 2012b). Further, a prospective cohort study involving 27 couples undergoing IVF treatment found that increasing urinary BPA concentrations in the

male, but not female partner, decreased the odds of a high embryo fragmentation score, suggesting the embryos were low quality for IVF treatments (Bloom et al. 2011b). Collectively, these studies indicate adverse associations of parental BPA levels on the development of early embryos *in vitro,* but further studies are required to determine whether the observed effects occur *in vivo*.

Although only two experimental studies have investigated the effects of BPA exposure on early embryo development *in vivo*, the results are intriguing. One study showed that high dose BPA given to pregnant C57BL/6 mice from GD0.5 to 3.5 delayed embryo development (Supplemental Material, Table S3) (Xiao et al. 2011). A more recent study of F1 female C57BL/6 Cast7 mice showed that low dose BPA exposure initiated prior to breeding and continuing through pregnancy disrupted the expression of imprinted genes in mid-gestation embryos and placentae (Susiarjo et al. 2013). Given the limited number of studies on the effects of BPA on embryo development *in vivo*, additional studies are needed prior to making firm conclusions about the effects of BPA on early embryos.

Placenta

Although epidemiological studies on BPA and the placenta have not been published, one experimental study suggests that both low and high doses of BPA increase plasma estradiol, testosterone, and corticotropin releasing hormone levels due to an increase in mRNA expression of corticotrophin releasing hormone and activation of protein kinase C ζ/λ and δ in the placenta (Supplemental Material, Table S3; Tan et al. 2013). A few *in vitro* studies indicate that BPA affects placental cell proliferation. After 24h or 48h of culture, BPA (1 to 10 μM) increased apoptosis and decreased proliferation of cultured human trophoblast cells from first trimester placentas, while BPA at 10 μM decreased cell viability (Morice at al. 2011). Similarly, after 24h,

BPA (87.7 nM to 8.77 µM) increased apoptosis in cultured human cytotrophoblasts (Benachour and Aris 2009). In addition, recent data from mouse studies suggest that BPA alters gene expression in the placenta (Susiarjo et al. 2013). Taken together, these *in vitro* data suggest that BPA may affect placental function, but additional animal and human studies are required to substantiate these data.

Pregnancy outcomes

Only one study has examined the association between BPA exposure and pregnancy outcome in humans. Specifically, a nested case control study of 60 pregnant women found a positive association between urinary BPA concentration and pre-term birth (Supplemental Material, Table S4; Cantonwine et al. 2010). Given the limited information on BPA and pregnancy outcomes in humans, more studies are needed before making firm conclusions about whether BPA exposure is association with adverse pregnancy outcomes in humans.

In contrast, several experimental studies have investigated the effects of BPA exposure on pregnancy outcomes in animal models such as mice and rats that are altrical species in which pups are born at a stage of development equivalent to mid-gestation in humans. Low dose BPA did not alter gestation length in gestationally and neonatally exposed mice (Supplemental Material, Table S5; Cabaton et al. 2011; Kobayashi et al. 2010), adult exposed mice (Tyl et al. 2008), or gestationally and neonatally exposed SD rats (Kobayashi et al. 2012). In other studies, low dose BPA exposure decreased the number of pregnancies and successful deliveries in gestationally exposed mice (Cabaton et al. 2011). BPA also decreased the percent of hatchings of chickens exposed *in ovo* to BPA (134 ng/kg) on day 4 on incubation (Yigit and Dagliogu 2010). Conversely, neither low nor high dose BPA affected the number of litters born to unexposed

female SD rats mated to gestationally exposed male rats (Thuillier et al. 2009), suggesting that maternal, but not paternal, BPA exposure may influence successful delivery of offspring.

Many experimental studies have reported that low dose BPA does not affect the number of live pups (Howdeshell et al. 2008; Kobayashi et al. 2010, 2012; Thuillier et al. 2009; Xi et al. 2011) or total number of delivered pups (Kobayashi et al. 2010, 2012; Nanjappa et al. 2012; Ryan et al. 2010; Tyl et al. 2008; Xi et al. 2011) in mice and rats. In a few studies, however, low dose BPA exposure decreased the number of live pups born to gestationally and neonatally exposed CD-1 mice (Cabaton et al. 2011) and Holtzman rats (Salian et al. 2009b). Low dose BPA also decreased the total number of pups born to gestationally and neonatally exposed CD-1 mice (Cabaton et al. 2011) Interestingly, in these studies, BPA acted differently from the positive control used (diethylstilbestrol: DES), suggesting the effects of BPA may differ from those of DES in CD-1 mice. Low dose BPA also decreased the total number of pups born to unexposed female Holtzman rats mated to neonatally and gestationally exposed male Holtzman rats (Salian et al. 2009a, 2009b). In these studies, BPA exposure decreased pup numbers after multiple litters, similar to DES used in the study. Collectively, these studies suggest that BPA exposure affects pregnancy outcomes in many, but not all studies depending on experimental protocol. Clearly, more studies are required to determine why results differ between studies and to determine if BPA exposure does indeed affect pregnancy outcomes in animal models.

Birth weight

Human studies of BPA and birth weight are equivocal (Supplemental Material, Table S4). In a cross-sectional study of 97 pregnant women, those women with serum BPA concentrations greater than 2.51 ng/mL had a higher risk for having low birth weight male neonates that were small for gestational age, compared to women with lower serum BPA concentrations. No

associations were found with female neonates (Supplemental Material, Table S4; Chou et al. 2011). These results are consistent with a retrospective cohort study that included 587 births in which maternal and paternal occupational exposure to BPA were both associated with low birth weight and small gestational size, but the association was stronger for maternal compared to paternal exposure (Miao et al. 2011b). Further, in a Dutch population-based prospective cohort study of 219 pregnant women, urinary BPA was positively associated with lower growth rates and head circumference (Snidjer et al. 2013). Similarly, in a birth cohort study of 757 Korean pregnant women participating in a Mothers and Children's Environmental Health study, BPA was associated with increased birth weight in males and neonatal length in females (Lee B et al. 2013). However, in a case-control study of 191 pregnant women, BPA had an inverse U-shape association with birth weight (Philippat et al. 2011). In a cross-sectional study of 40 pregnant women, urinary BPA levels at delivery were not associated with birth weights of the offspring (Padmanabhan et al. 2008). Further, in a prospective cohort study of 404 mother-infant pairs, BPA exposure was not associated with increased birth weight (Wolff et al. 2008b). Given some of these discrepant results, future studies should be conducted that assess whether BPA exposure is associated with birth weight.

In previous work, gestational exposure to low doses of BPA did not alter the birth weight of mice or rats. However, recent animal studies suggest that the effect of BPA on offspring birth weight is dose dependent (Supplemental Material, Table S5). High dose BPA increased the weight of offspring born to gestationally exposed mice (Tyl et al. 2008). Conversely, low dose BPA decreased the weight of offspring born to neonatally exposed mice (Nah et al. 2011). However, at doses below the EPA reference dose (50 μg/kg), BPA did not affect the weight of offspring born to gestationally and neonatally exposed mice (Kobayashi et al. 2010) or rats (Howdeshell et

al. 2008; Kobayashi et al. 2012; Ryan et al. 2010; Nanjappa et al. 2012), suggesting that BPA has a specific dose effect on birth weight of offspring.

Sperm production and quality

Human studies of the effect of adult exposure to BPA on sperm quality are very limited (Supplemental Material, Table S6). In studies of occupationally exposed men and men recruited from an infertility clinic, higher urinary BPA levels were associated with a decrease in sperm count and motility (Li DK et al. 2011; Meeker et al. 2010a). However, in a study of fertile men, urinary BPA concentration was not associated with changes any semen parameters, although a significant association between urinary BPA levels and markers of free testosterone was observed (Mendiola et al. 2010). Although the results of these few epidemiological studies are consistent, there is insufficient evidence to draw conclusions about the association between BPA and semen quality in humans due to the limited number of studies to date.

The earliest experimental studies of the effects of BPA on sperm suggested adverse effects on spermatogenesis in the adult following either prenatal or early postnatal exposure (reviewed in Richter et al. 2007). Subsequent experimental studies have provided further evidence that prenatal and early postnatal exposure adversely affect spermatogenesis and have provided new evidence that exposure in adult rodents affects sperm quality (Supplemental Material, Table S7). In rodents, exposure during the time of testis development, either during gestation or in the early postnatal period, or to young adult males, has been associated with a range of adverse effects in the adult testis. However, variability in exposure timing, species and strains, endpoints, and life stage makes a simple analysis of the data difficult. Despite the variation in study design, several findings have emerged repeatedly, including decreased sperm counts and/or increased apoptotic

cells within the seminiferous tubules and decreased sperm motility, alterations in hormone levels and/or steroidogenic enzymes, and evidence of sperm DNA damage.

The evidence that BPA exposure adversely affects sperm production and quality in the adult rodent is supported by data from recent studies (Supplemental Material, Table S7). Gestational low dose BPA exposure decreased the number of elongated spermatids present in seminiferous tubules in pubertal ICR mice (Okada and Kai 2008) and decreased sperm counts in Holtzman rats (Salian et al. 2009a). Similarly, both low and high dose BPA exposure during early postnatal development or around the time of puberty increased apoptosis and/or decreased spermatogenesis in male mice and rats (Li Y et al. 2009; Liu et al. 2013; Qiu et al. 2013; Wang et al. 2010). Further, low dose BPA exposure in adult rats exposed for either 6 or 14 days increased apoptosis and decreased sperm counts (Jin et al. 2013; Tiwari and Vanage 2013).

In addition, a number of studies using various routes of exposure (oral and subcutaneous) as well as different exposure times (embryonic, fetal, perinatal, and adult) have shown that low dose BPA impairs sperm motility in rats and mice (Supplemental Material, Table S7; Dobrzynska and Radzikowska 2013; Minamiyama et al. 2010; Salian et al. 2009a; Tainaka et al. 2012; Tiwari and Vanage 2013). Interestingly, one study demonstrated that the BPA-induced decrease in rat sperm motility was prevented by co-administration of the antioxidant, n-acetyl cysteine (Minamiyama et al. 2010), suggesting that impaired sperm motility may be related to increases in reactive oxygen species. As reviewed recently, antioxidant use has been suggested to increase sperm quality in men and increase pregnancy rates of infertile couples (Ross et al. 2010).

Spermatogenesis is dependent upon androgens, and changes that impact the endocrine environment of the testes have the potential to adversely impact sperm production and quality.

Endocrine-related changes have been reported in several experimental studies: gestational only, as well as continuous gestational-neonatal exposure studies in rodents have reported evidence of either impaired steroidogenesis, a decrease in the number of steroid receptors, or a decrease in *Star* expression (Supplemental Material, Table S7). However, the most compelling data come from rat studies of males exposed in the postnatal/adult period, where most reported a decrease in testosterone levels and/or steroidogenic enzymes in low or high dose BPA exposed males compared to controls (Castro et al. 2013; D'Cruz et al. 2012a; El-Beshbishy et al. 2012; Jin et al. 2013; Nakamura et al. 2010; Wu et al. 2011). Only a single study in ICR mice reported no change in testosterone levels in low dose BPA exposed males compared to controls, despite the fact that the exposed males had impaired spermatogenesis (Okada and Kai 2008).

In addition to causing endocrine changes, BPA exposure has been shown to cause sperm DNA damage (Supplemental Material, Table S7). Consistent with studies before 2007 (Chitra et al. 2003; Kabuto et al., 2003; Kabuto et al., 2004), recent studies show that continuous low dose BPA exposure induces DNA breaks and the production of reactive oxygen species (ROS) in mice and rats (Supplemental Material, Table S7; Anjum et al. 2011; Dobrzynska and Radzikowska 2013; Fang et al 2013; Liu et al. 2013; Minamiyama et al. 2010; Rashid et al. 2009; Wu et al 2013). Low dose oral BPA exposure for 6 days also induces sperm DNA damage (Tiwari and Vanage 2013). Similarly, low dose oral BPA exposure decreases testicular glucose levels and the expression and translation of glucose transporter-8 in spermatocytes and spermatids and it increases testicular hydrogen peroxide levels (D'Cruz et al. 2012a, 2012b) in Wistar rats and oxidative stress in Sprague-Dawley rats (Qiu et al. 2013).

Unfortunately, the relevance of the doses used in the experimental studies to human exposure is not certain because internal or serum BPA levels were not determined in the studies.

Nevertheless, these data add to the evidence that BPA exposure in the adult rodent adversely impacts the testis and sperm quality at levels below the LOAEL of 50 mg/kg. Although similar reproductive effects have been reported in multiple studies, a few studies failed to find any adverse reproductive effects in rodent models following gestational and neonatal BPA exposure (Supplemental Material, Table S7; Howdeshell et al. 2008; LaRocca et al. 2011; Tyl et al. 2008; Kobayashi et al. 2010). These discordant findings may be related to a variety of factors, including dose, exposure route, internal dose, timing, and selected endpoints. Nevertheless, commonalities among studies finding no adverse effects raise several concerns. First, two of the four studies used the same inbred mouse strain, C57BL/6 (LaRocca et al. 2011; Kobayashi et al. 2010), suggesting that this strain may be insensitive or less sensitive to the BPA effects on sperm than other strains. Second, dietary exposure has been used in several "no effect" studies, but was not used in any studies reporting adverse effects. These results add to the concern about the role route of exposure plays in BPA studies and substantiates calls to replace exposure route with measurements of internal dose. Indeed, recent studies demonstrate that the route of BPA administration results in markedly different ratios of unconjugated (bioactive) to conjugated BPA and provide evidence that much of human exposure must be from non-oral routes (Gayrard et al 2013, vom Saal et al 2014). Lastly, two of the studies (Howdeshell et al. 2008; Tyl et al. 2008) focused on endpoints such as body/organ weight, which alone may not accurately measure effects on male fertility. Indeed, some studies reporting detrimental effects, e.g., increased germ cell apoptosis or reduction in sperm counts, did not find significant changes in testis or epididymal weight (Supplemental Material, Table S7). Nevertheless, these negative findings underscore the need for comparative experimental studies involving different strains of animals and routes of exposure. In addition, assessing the importance of effects during different life

stages is important, particularly because Newbold et al. (2009) identified abnormalities in middle age that were not seen in young adult female rodents.

Steroidogenesis in males

Three epidemiological studies have investigated the association between BPA exposure and serum hormone levels in men (Supplemental Material, Table S6). In one study of 307 Italian men participating in the INChianti study, a prospective, population based study of adults living in Chianti, Italy, increased urinary BPA concentrations were associated with increased serum testosterone, but not estradiol levels (Galloway et al. 2010). This observation was not supported by two cross-sectional studies of 167 and 302 fertile men, which reported no correlation between BPA exposure and testosterone levels (Meeker et al. 2010b, Mendiola et al. 2010). However, these studies found associations between urinary BPA concentrations and decreased free androgen index (FAI), the ratio of FAI to luteinizing hormone, and the ratio of free testosterone to luteinizing hormone in male partners of pregnant women (Mendiola et al. 2010). The study also found an association between high BPA concentrations and increased serum levels of follicle-stimulating hormone, but decreased levels of inhibin B, the ratio of follicle-stimulating hormone to inhibin B, and the ratio of estradiol to testosterone (Meeker et al. 2010b). These data suggest the BPA can alter steroid hormone pathways in men, but because of limited association studies and likely multifactorial responses produced by BPA, more studies are needed to determine how BPA affects steroidogenesis. Further, because most studies conducted prior to 2007 focused on infertile men, it is important to conduct studies on fertile men to confidently determine if BPA affects male steroidogenesis and whether findings in infertile men can be extrapolated to the general population.

Experimental studies also indicate that BPA exposure decreases hormone levels in male animals, but the data are discordant (Supplemental Material, Table S7). Low dose BPA exposure decreased testosterone levels in gestational through postnatally exposed CD-1 mice (Xi et al. 2011), but not in adult C57BL/6 mice exposed *in utero* (LaRocca et al. 2011). Low dose BPA decreased testosterone levels in gestationally or neonatally exposed Holtzman rats (Salian et al. 2009a, 2009b), adult exposed albino rats (El Beshbishy et al. 2012), and adult exposed Wistar rats (D'Cruz et al. 2012a). Conversely, gestational and neonatal low dose BPA exposure did not affect testosterone levels in LE rats (Howdeshell et al. 2008) or SD rats (Kobayashi et al. 2012; Qiu et al. 2013). However, while adult exposure did not decrease testosterone in Wistar rats, low dose BPA decreased dihydrotestosterone levels (Sánchez et al. 2013). Mechanistic underpinnings for the ability of BPA to inhibit testosterone levels may be found in the fact that BPA decreases expression of steroidogenic enzymes (Horstman et al. 2012; Nakamura et al. 2010; Qiu at al. 2013; Xi et al. 2011) and follicle-stimulating hormone levels, which are required to directly and indirectly stimulate steroidogenesis (Salian et al. 2009a, 2009b). Collectively, these data indicate that BPA exposure decreases sex steroid hormone levels in male rodents, but strain and species as well as other confounders (e.g., time and route of exposure, and age at analysis) may modulate the sensitivity to BPA effects.

Because Leydig cells are responsible for the majority of sex steroid hormone production in males, experimental studies have been designed to examine whether BPA directly affects the Leydig cells. In pubertal Wistar/ST rats, continuous exposure to high dose BPA decreased cell numbers and expression of steroidogenic enzymes in the Leydig cells (Nakamura et al. 2010). Conversely, low dose BPA increased Leydig cell numbers in gestationally and neonatally exposed LE rats in adulthood by up-regulating mitogenic factors (Nanjappa et al. 2012). Though

BPA exposure increased Leydig cell proliferation, it did not change circulating testosterone levels. In fact, low dose BPA decreased the expression of steroidogenic enzymes and testosterone production by Leydig cells isolates from adult exposed males and cultured *in vitro* (Nanjappa et al. 2012). Similarly, BPA decreased testosterone levels in human, mouse, and rat fetal testes cultured *in vitro* (N'Tumba-Byn et al. 2013). In another study, low and high dose BPA and increased Leydig cell numbers without affecting serum testosterone-levels in gestationally exposed SD rats in adulthood (Thuillier et al. 2009). Collectively, these data strongly suggest that BPA negatively affects gonadal function through changes in steroid synthesizing cells/enzymes, which then affect steroid synthesis and circulating steroid levels.

Anogenital distance and cryptorchidism

Disruptions in testicular testosterone production during development may lead to phenotypic abnormalities after birth such as a shortened anogenital distance (AGD) and undescended testes. One retrospective occupational study of 587 Chinese children found a dose-dependent association between parental occupational exposure to BPA and shortened AGD in male offspring (Supplemental Material, Table S4; Miao 2011b). This inverse correlation was strengthened with increasing maternal BPA concentrations (Miao et al. 2011a). Interestingly, the concentrations of BPA in cord blood samples were not correlated with cryptorchidism in a matched case-control study of 152 newborn males (Fenichel et al. 2012). While these studies suggest that BPA exposure may be associated with shortened AGD and undescended tests, it is important to confirm these findings in other epidemiological studies.

In several animal studies, BPA exposure did not affect AGD, but it did influence testes descent (Supplemental Material, Table S7). In four recent studies, gestational or gestational and neonatal low dose BPA exposure did not alter AGD in male rodents (Howdeshell et al. 2008; Kobayashi

et al. 2010, 2012; LaRocca et al. 2011). In one other recent study, gestational exposure to high dose BPA decreased absolute AGD in F1 CD-1 males at birth, though not in the F2 generation and not when normalized to relative body size (Tyl et al. 2008). Interestingly, high dose BPA delayed preputial separation and increased the incidence of treatment-related, undescended testes in F1 and F2 offspring (Tyl et al. 2008). Notably, the doses used in this study were very high and not physiologically relevant to human exposure. Collectively, these data suggest that BPA exposure may not affect AGD in animal models, but it may affect other developmental aspects such as undescended testes.

Male urinary tract

To date, no epidemiological studies have reported on whether BPA exposure is associated with alterations in the male urinary tract and only a few experimental studies have evaluated the effects of BPA on the male urinary tract (Supplemental Material, Table S7). In adult rat prostates, low and high dose BPA exposure adversely increased clusterin levels and expression of aromatase, while decreasing 5 alpha reductase type 1 and 2 levels (Castro et al. 2013; De Flora et al. 2011; Sánchez et al. 2013). Interestingly, in the De Flora and Castro studies (Castro et al. 2013; De Flora et al. 2011), BPA exposure increased the plasma estradiol to testosterone ratio, which has been implicated in the development of benign prostate hyperplasia (Nicholson et al. 2011; Nicholson and Ricke 2013). Further, gestational exposure to low dose BPA increased androgen receptor, *Esr1*, aromatase, and estrogen-related receptor gamma gene expression in the mouse prostate (Arase et al. 2011).

Recent studies also indicate that BPA exposure plays a role prostate pathogenesis in animal models. Neonatal low dose BPA exposure increased the incidence of the precursor for prostate cancer, prostate intra-epithelial neoplasia in adulthood in rats (Prins et al. 2011). Further, in rats

with induced prostatic hyperplasia, high dose BPA increased prostate gland mass and increased relative weight of the dorsolateral prostate lobe (Wu et al. 2011). High dose BPA exposure also increased epithelial cell heights of the ventral prostate and dorsolateral prostate lobes (Wu et al. 2011). Lastly, neonatal exposure to low dose BPA led to transient and permanent hypo-methylations in the rat epigenome implicated in the manifestation of prostatic carcinogenesis (Tang et al. 2012). Collectively, these studies support the concept that both low and high doses of BPA promote changes in the steroidogenic pathways and morphology of the prostate, which may in turn affect homeostasis and pathogenesis.

Currently, no information is available about whether BPA exposure causes benign urologic disease. However, studies have shown that estrogens and estrogen receptor pathways negatively affect the lower urinary tract (Nicholson et al. 2012; Ricke et al. 2008; Wang et al. 2007; Willingham and Baskin 2007), supporting a potential role for estrogen-like molecules, including BPA, in the manifestation of urological diseases. Future experimental studies evaluating the effects of BPA on the lower urinary tract as well as epidemiological studies in humans are to gain insight into the role BPA plays in disease processes in the lower male urinary tract.

Puberty and sexual receptivity

Two epidemiological studies investigated the effect of BPA on puberty and measured BPA levels in girls at similar ages (Supplemental Material, Table S2). In these studies, one of 1151 girls aged 6-8 years and another of 192 girls aged 9 years, BPA exposure was not associated with accelerated breast or pubic hair development (Wolff et al. 2008a, 2010). Additionally, BPA exposure was not associated with precocious puberty in a study of 82 patients with precocious puberty and 32 patients without precocious puberty (Lee SH et al. 2013). However, in a study of 110 girls with precocious puberty and 100 girls without precocious puberty, BPA was associated

with increased uterine and ovarian volume (Qiao et al 2010). These studies suggest that BPA exposure may not be associated with onset of puberty in girls, but given the limited number of studies, these results should be confirmed in future studies.

In animal models, the effects of BPA on factors such as puberty onset and sexual receptivity are equivocal (Supplemental Material, Table S8). In one study, low dose BPA did not affect the timing of puberty onset as measured by vaginal opening in gestationally and neonatally orally exposed LE rats (Ryan et al. 2010). Conversely, low and high dose BPA exposure accelerated vaginal opening in neonatally exposed ICR mice, SD rats, and LE rats (Adewale et al. 2009; Fernandez et al. 2009; Nah et al. 2011). Low dose BPA also decreased the time spent in estrus (Fernandez et al. 2009; Nah et al. 2011) in neonatally exposed ICR mice and SD rats. However, low dose BPA did not affect estrous cyclicity in gestationally and neonatally exposed CD-1 mice and LE rats (Adewale et al. 2009; Tyl et al. 2008). Further, low dose BPA had no effect on lordosis behavior in gestationally and neonatally exposed LE rats (Ryan et al. 2010) or neonatally exposed female LE rats (Adewale et al. 2009). Collectively, these studies indicate that the effects BPA exposure on the onset of puberty and sexual receptivity in animal models are unclear and likely differ depending on strain and species. Further studies should investigate how strain and animal models influence puberty onset and sexual receptivity.

Sexual dysfunction

Only a few studies have examined the association between BPA exposure and sexual dysfunction in epidemiological or experimental studies. One cross-sectional study of 425 men occupationally exposed to BPA had an increased risk of self-reported impaired sexual abilities compared to 284 unexposed men (Supplemental Material, Table S6; Li DK et al. 2010). Some animal studies have also shown that BPA may impair sexual ability (Supplemental Material,

Table S8). Low dose BPA increased the time taken for copulation in neonatally and gestationally exposed Holtzman rats (Salian et al. 2009a, 2009b) and increased latency to insemination in perinatally exposed CF-1 mice (Decatanzaro et al. 2013). Low dose BPA also decreased intromission and ejaculations of unexposed CF-1 mice mated to perinatally exposed females (Decatanzaro et al. 2013). However, low or high dose BPA did not affect the time taken for copulation in gestationally and neonatally exposed adult CD-1 mice or their offspring (Tyl et al. 2008). Given the limited number of studies conducted on BPA and sexual function and the equivocal nature of the results, it is not possible to make firm conclusions on the effects of BPA on sexual function. However, it is important to note that all, but one study conducted to date, suggest that BPA negatively impacts sexual function.

Conclusions

Below we summarize the strength of the evidence for associations between BPA and adverse reproductive outcomes based on literature published from 2007-2013. The data presented in this review build on the overall conclusions of the expert panel report in 2007 (vom Saal et al. 2007) that the widespread effects of BPA in experimental animal studies are a concern for overall human health and may be involved in human reproductive disease. Similar to the 2007 expert panel report, we considered the evidence to be strong when multiple studies in multiples species indicated a similar effect of BPA on a reproductive tissue or endpoint, even if concordance was not 100% across all studies given that species and strain differences can lead to differences in dose response and magnitude of effect. These conclusions, however, are not to be considered definitive without further investigation, especially with the gaps in clear results detailed throughout the review. In the experimental studies, strong, definitive conclusions often were difficult because study designs were so different. Experimental studies rarely utilized the same

doses, timing, positive controls, and exposure routes to compare the effects of BPA on exposure among animal strains and species. However, one unifying strength of these studies related to human health is that the majority of work evaluated in this review used BPA doses below the LOAEL. In the epidemiological studies, strong conclusions were difficult to determine because of study design and exposure parameters. For example, exposure assessments in the majority of human studies rely on a single urine sample, which may introduce exposure misclassification and attenuate associations if they are present. Given the continuous and variable exposure to BPA, a single urine sample may not represent longer term exposure or exposure in the relevant etiological window. Finally, a majority of the human studies were cross-sectional, making it difficult to discern the temporal relationship of exposure with response. These limitations affect the interpretation of human studies. Future human studies need to consider improvements in exposure assessment to represent longer-term BPA exposure assessed at etiologically relevant window(s). Given recent data, it will be critical in future studies to assess effects of different routes of exposure and to analyze effects at different stages in the life of the exposed individual. Additionally, for experimental studies, the addition of positive controls should be considered essential.

However, given the data included in this review, we have drawn some insights and conclusions that add to the conclusions drawn based on reviews of the BPA literature prior to 2007, categorized by the strength of evidence presented.

(1) Strong evidence exists that:

- BPA is an ovarian toxicant in animal models and women. It adversely affects the onset of meiosis in ovaries from both animal models and humans, interferes with germ cell nest breakdown in animal models, accelerates follicle transition in several

animal species, alters steroidogenesis in multiple animal models and women, and
reduces oocyte quality in animal models and women undergoing IVF.

- BPA is a uterine toxicant in animal models because it impairs uterine endometrial
cellular proliferation, decreases uterine receptivity, alters gene expression, and
increases implantation failure in several strains/species. However, human studies
have not adequately addressed these endpoints.

- BPA is a prostate toxicant in animal models, impairing the steroidogenic capacity and
altering dorsal and ventral lobe morphology, potentially leading to prostate
pathogenesis. Human studies, however, are lacking.

- The effects of BPA on the reproductive system are variable and evident at doses
below the LOAEL of 50mg/kg and the proposed safe level of 50μg/kg/d.

(2) Limited evidence exists that:

- Relative to impact of BPA on birth rate, birth weight and length of gestation, human
data on birth weight are inconsistent; although animal studies suggest such an
association, and there are limited human studies on gestational length and pre-term
birth.

- BPA exposure is associated with hyperandrogenism, such as in PCOS in women.
However, data in rodent models are not supportive of the development of human
PCOS symptoms.

- BPA is a testicular toxicant in animal models because it decreases sperm quality,
motility, causes oxidative stress, and alters steroidogenesis. Human data, however,
are inconsistent.

- BPA is associated with impaired implantation in women undergoing IVF. The associations between BPA and implantation failure in women may be due to the effects of BPA on the embryo, uterus, or both.

- BPA exposure in male rats is associated with implantation failure in non-exposed female rats. Human data, however, are lacking.

- BPA is associated with sexual dysfunction among men exposed to high occupational levels and in experimental studies on males.

(3) There is insufficient evidence to draw conclusions regarding effects of BPA on the oviduct, placenta, and pubertal development.

(4) Future studies need to:

- Consider the critical period of differentiation of the organ system in question and the reproductive life span of the animal model or human.

- Use continuous exposure to BPA in view of the ubiquitous and continuous exposure to BPA in humans.

- Target internal dose levels of BPA that are achieved by human exposures.

- Recognize the potential interaction of BPA with other hormone altering chemicals and life style factors such as diet and stress.

- Distinguish organizational (permanent) vs. activational (transient) effects of BPA.

- Determine whether pre-term and maternal morbidities (e.g., pre-eclampsia, gestational diabetes) as well as paternal factors modify or mediate the effects of BPA on the reproductive system.

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